AD_________________

Award Number: DAMD17-02-1-0396

TITLE: Differential Control of ErbB2 Surface Expression in Breast Cancer Cells by an Alternatively Spliced Form of ERBIN

PRINCIPAL INVESTIGATOR: Kathryn H. Ching

CONTRACTING ORGANIZATION: Georgetown University
Washington, DC 20007

REPORT DATE: May 2005

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)* 01-05-2005	**2. REPORT TYPE** Annual Summary	**3. DATES COVERED** *(From - To)* 1 May 2002 - 30 Apr 2005

4. TITLE AND SUBTITLE Differential Control of ErbB2 Surface Expression in Breast Cancer Cells by an Alternatively Spliced Form of ERBIN	**5a. CONTRACT NUMBER**
	5b. GRANT NUMBER DAMD17-02-1-0396
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S) Kathryn H. Ching	**5d. PROJECT NUMBER**
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Georgetown University Washington, DC 20007	**8. PERFORMING ORGANIZATION REPORT NUMBER**
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012	**10. SPONSOR/MONITOR'S ACRONYM(S)**
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Abstract follows.

15. SUBJECT TERMS
Protein trafficking, growth factor receptor, MVB

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT U	**b. ABSTRACT** U	**c. THIS PAGE** U	UU	18	**19b. TELEPHONE NUMBER** *(include area code)*

ABSTRACT

Snf7p and Vps20p are involved in yeast MVB structure, formation and function. We cloned and characterized 3 human homologues of yeast Snf7p, designated hSnf7-1, hSnf7-2 and hSnf7-3, and a single human Vps20p homologue, hVps20, that may have similar roles in humans. Immunofluorescence studies showed that hSnf7-1 and hSnf7-3 localized in large vesicular structures that also co-localized with late endosomal structures induced by overexpressing an ATPase-defective Vps4-A mutant. Overexpressed hVps20 showed an endosomal membrane-staining pattern, and co-expression of hVps20 with Snf7-1 dispersed the large Snf7-staining vesicles. Interestingly, overexpression of both hSnf7 and hVps20 proteins induced a post-endosomal defect in cholesterol sorting. To explore possible protein–protein interactions involving hSnf7 proteins, we used information from yeast genomic studies showing that yeast Snf7p can interact with proteins involved in MVB function. Using GST-capture with several mammalian homologues of such yeast Snf7p-interacting proteins, we found that all three hSnf7s interacted with AIP1, a mammalian Bro1p-containing protein involved in cellular vacuolization and apoptosis. Mapping experiments showed that the N-terminus of AIP1 containing both a Bro1 and an α-helical domain were required for interaction with hSnf7-1, Snf7-1 did not interact with another human Bro1-containing molecule, rhophilin-2. Co-immunoprecipitation experiments confirmed the *in vivo* interaction of hSnf7-1 and AIP1. Immunofluorescence experiments showed that hSnf7-1 recruited cytosolic AIP1 to the Snf7-induced vacuolar-like structures. These results suggest that mammalian Vps20, AIP1 and Snf7 proteins and a new component (UEV3) may play roles in MVB function.

Table of Contents

I. Introduction

Increased numbers of certain growth factors (e.g. ErbB2/Her2) occur in many breast cancer tumor cells. One of the major pathways involved in removing growth factor receptors from the cell surface following their activation and endocytosis is that of the Multivesicular body (MVB) pathway (Fig. 1). Following their activation and internalization, cell surface receptors are ubiquitylated and targeted to multivesicular endosomes, which are regulated by a complex set of cellular proteins. Ultimately, the amount and activity of these MVB components determines whether the cargo is recycled to the cell surface or transported to the lysosomes where degradation occurs.

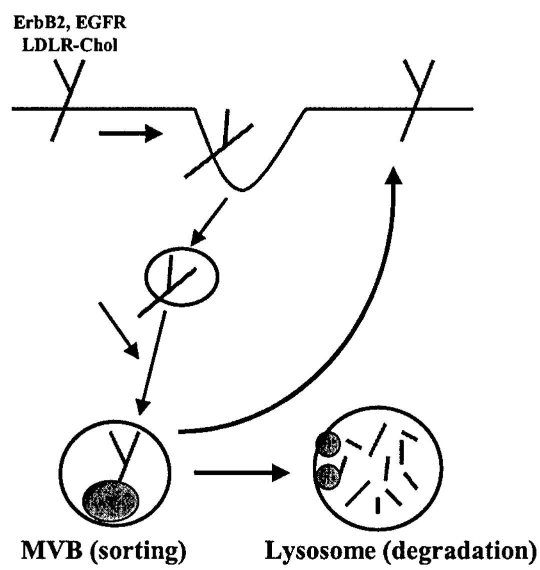

Figure 1. **Protein sorting occurs in the MVB pathway.** Following ligand binding, many receptors (e.g. ErbB2) are down-regulated. Endocytosed receptors enter the multivesicular body pathway where they are then targeted for either lysosomal degradation or recycling back to the cell surface.

Cellular proteins can be endocytosed from the plasma membrane via early endosomes. The subsequent sorting of these proteins for recycling to the plasma membrane or for degradation in the lysosomes occurs in a complex, multi-step process during which internal vesicles of late endosomes are relocalized to multivesicular bodies (MVB) (1). More than 20 yeast proteins, the class E vacuolar protein-sorting (*vps*) proteins, are important for endosomal sorting, as shown by *vps* mutants that fail to efficiently transport newly synthesized hydrolases efficiently to a vacuole (2). Yeast studies have identified three protein complexes termed ESCRT-I, -II and-III (endosomal sorting complex required for transport) involved in the sequential processing of ubiquitinylated endocytosed membrane proteins for inclusion into the MVB pathway (3,4). ESCRT-I is involved in binding ubiquitinylated cargo and activates ESCRT-II, which then assembles the ESCRT-III complex on internal endosomal membranes prior to the formation of a new vesicle within the MVB cargo (3,4). The yeast ESCRT-III complex is composed of at least four proteins, Snf7p (also known as Vps32), Vps2p, Vps20p and Vps24p (4). Mutations in each of these four yeast genes causes defects in the transition from the late endosome to the MVB, and expression of these mutants results in failure to deliver target proteins to the yeast vacuole. The Vps20p-Snf7p subcomplex is associated with the late endosome membrane, and the Vps2p-Vps24p subcomplex appears to bind the membrane-bound Vps20p-Snf7p subcomplex.

The mammalian MVB pathway has only recently been studied in detail, and many of the components show marked homology to components of the yeast pathway. The recent interest in the human MVB pathway stems from its involvement in the release of several viruses from infected cells, but also because the pathway influences cell signaling by controlling the levels of cell surface receptors at the plasma membrane and the availability of other signaling components, such as intracellular kinases. One of the first components involved in this process, TSG101, directly binds a PTAP motif, a short tetrapeptide sequence in the late domain GAG protein of several viruses including HIV (5), and Ebola (6-8). Mapping experiments showed that the UEV, domain located in the N-terminus of TSG101, was responsible for binding this tetrapeptide sequence (9). Subsequent studies found TSG101 normally interacts with PTAP and related PSAP motifs in cellular MVB proteins, such as Hrs (10,11) and AIP1 (12). AIP1, in turn, interacts with additional MVB cellular components including human CHMP4 proteins (also known as hSnf) (12-16) and induces vacuolization possibly via its interactions with endophilins (17). The interaction of AIP1 with CHMP4 proteins may allow it to regulate the ESCRT-III complex, since these proteins can recruit AIP1 into endosomal/lysosomal structures (15,16). Here we have characterized one and three human homologs of the yeast late endosomal ESCRT-III complex proteins, Vps20p and Snf7p. Additional ongoing studies characterizing a potential novel component, UEV3, of ESCRT-I, are also presented.

II. Body

This study originally proposed to uncover the function of Erbin in ErbB2 receptor trafficking and cell signaling. Since this original grant proposal was accepted in July 2000, it has taken a major turn in focus (change in aims and topic approved by DOD material command) and also involved a change in the PI. This final report reflects these revised aims, which are related to protein trafficking through the multivesicular body pathway. Some of the work described below has already been published (15).

Our studies were initiated by searching for human proteins homologous to yeast Snf7p, a critical component of MVB function. Clones were identified by a TBLASTN search of the EST NCBI database using the yeast Snf7p protein sequence as a query. In contrast to yeast, three human Snf7 homologs were identified (Fig. 2). Plasmids containing cDNAs for these human proteins, designated hSnf7-1, hSnf7-2 and hSnf7-3, were obtained from the IMAGE consortium, sequenced and confirmed to encode proteins of 222, 224 and 234 amino acid residues, respectively (Fig. 2).

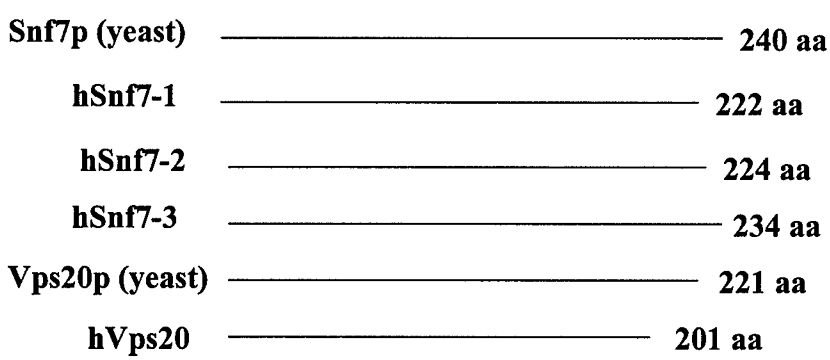

Figure 2. Structure of hSnf7 and hVps20. Human EST clones were obtained from the IMAGE consortium and sequenced. hSnf7-1, hSnf7-2 and hSnf-73 share 49, 50 and 49% sequence similarity, respectively, with yeast Snf7p. *In silico* secondary structure analysis revealed that all three human Snf7 proteins have amino acid sequences that probably fold, similar to Snf7p, into coil-coiled secondary structures. Similarly, yeast Vps20p was used as a query to identify and obtain a human Vps20 clone. The 201amino acid clone identified shared 44% sequence similarity with yeast Vps20p.

In light of these findings we have focused our attention on determining the function of human Snf-like proteins. Using indirect immunofluorescence, we looked for the cellular distribution and biological effect of overexpressed N-terminal FLAG epitope-tagged Snf7. We expressed epitope-tagged hSnf7-1 and hSnf7-3 in HeLa cells and examined their localization by immunofluorescence. Both N-terminal epitope-tagged hSnf7-1 (Fig. 3A and B) and hSnf7-3 (Fig. 3C and D) localized to vesicular-like structures in HeLa cells, some of which are unusually large. Cells coexpressing hSnf7-1 and hSnf7-3 showed complete colocalization to these large vesicular structures (Fig. 3E and F).

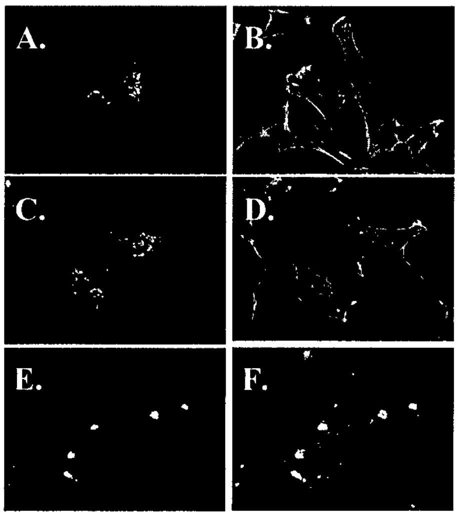

Figure 3 Expression of hSnf7-1 and hSnf7-3 in HeLa cells
Cells were transfected with either N-terminal Myc-tagged hSnf7-1 (**A** and **B**) or Flag-tagged hSnf7-3 (**C** and **D**) or cotransfected with Myc-tagged hSnf7-1 and Flag-tagged hSnf7-3 (**E** and **F**). Twenty-four hours after transfection, the cells were fixed, permeablized, and stained. hSnf7-1 (**A**) and hSnf7-3 (**C**) were detected with mouse monoclonal anti-Myc and anti-FLAG monoclonal antibodies, respectively and followed by staining with a FITC-conjugated goat anti-mouse antibody. F-actin was detected using Texas Red-conjugated phalloidin (**B** and **D**). In the cotransfected cells a mouse anti-Myc monoclonal antibody was used to detect hSnf7-1 protein (**E**), while a rabbit anti-FLAG polyclonal was used to stain for hSnf7-3 protein (**F**).

To identify the nature of these large hSnf7-containing vesicles, we used several different markers for vesicles involved in protein trafficking including an anti-EEA1 antibody, a marker for early endosomes, and lysotracker, a marker of late endosomes. While HeLa cells expressing hSnf7-3 showed the large vesicles in the perinuclear region in a similar location with EEA1 staining endosomes , these vesicles did not significantly colocalize (data not shown).

Although we proposed to test whether expression of human Snfs regulated EGF/EGFR trafficking, a recent study was published showing this was the case (16). Instead, we examined whether cholesterol trafficking was altered in the Snf7-expressing cells because the overexpression of two different human Snf7 proteins induced vesicular structures that morphologically resembled late endosomes/lysosomes induced by a Vps4-A mutant that is known to disrupts cholesterol trafficking (18). As might be predicted, cells showing accumulation of hSnf7 vesicular structures showed a marked enrichment for cholesterol in these same structures as detected by filipin (a marker of cholesterol) staining (Fig. 4A and B). This was in contrast to neighboring untransfected cells that showed normal cholesterol staining in much smaller punctuate structures (Fig. 4A and B). Unexpectedly, a similar, but less dramatic result was also observed when hVps20 was expressed in cells (Fig. 4C and 5D). Specifically in cells expressing a high level of hVps20 as shown in Figure 5C and 5D, a marked accumulation of large structures staining for cholesterol was observed (Fig. 4D). Furthermore, cells coexpressing both hSnf7 and Vps20 proteins also showed the accumulation of large vesicular structures containing cholesterol (data not shown). Taken together these results suggest

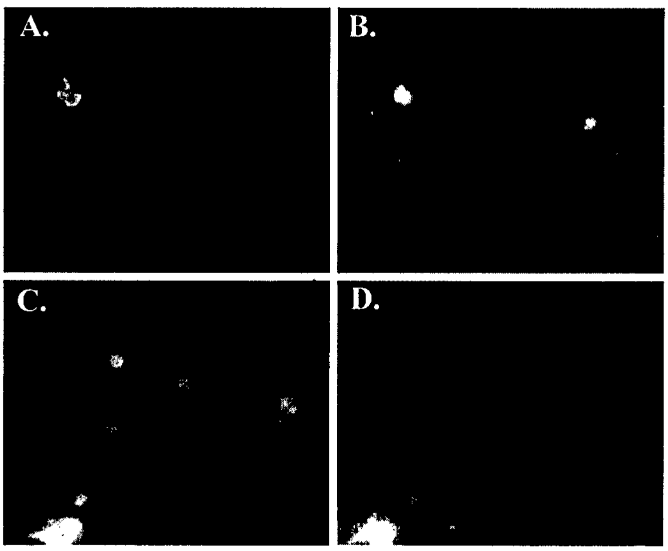

Figure 4 hSnf7 and hVps20 expression alters cholesterol trafficking
Cells were transfected with either FLAG-tagged hSnf7-3 (**A** and **B**) or C-terminal Myc-tagged hVps20 (**C** and **D**). Twenty-four hours after transfection, the cells were fixed, permeablized, and stained for FLAG-tagged hSnf7-3 (**A**) or Myc-tagged hVps20 (**C**). Flipin was used to stain cholesterol (**B** and **D**).

that hSnf7 proteins and hVps20 function in cholesterol trafficking and likely regulate the trafficking of other cell surfaces receptors.

In large scale yeast two-hybrid screens, Snf7p was found to interact with multiple proteins including Vps4p (19,20), the calpain protease Rim13p (20) and a Bro1 domain-containing protein, Rim20p (20). To determine if the mammalian counterparts of these yeast proteins have similar interaction abilities, we used an affinity-capture approach. In addition, we tested whether hVps20 might interact directly with hSnf7 proteins. For these experiments, we produced epitope-tagged proteins in mammalian cells including: hVps20; Vps4-A, a rat homolog of Vps4p (21), and AIP1, a human Bro1 domain-containing homolog of yeast Rim20p (22,23). **Western blot analysis of Cos1 cells transfected with hVps20, Vps4-A, PALBH and AIP1 mammalian expression vectors revealed that all of these proteins were expressed and migrated at approximately 35, 55, 110 and 90 kDa, respectively as expected for their predicted molecular weights (Fig. 5).** We tested the ability of recombinant GST-hSnf7-1, immobilized to glutathione beads, to bind these epitope-tagged proteins in Cos1 cell extracts. We found that AIP1 interacted strongly with GST-hSnf7-1 but not with GST (Fig. 5). While hVps20 or Vps4-A, were expressed at relatively high levels, they did not significantly interact with immobilized GST-hSnf7-1 (Fig. 5). Additional studies showed that both GST-hSnf7-2 and GST-hSnf7-3 also interacted with AIP1 but not Vps4-A (data not shown). The inverse experiments using GST-Vps4-A and GST-hVps20 left immobilized on glutathione beads also failed to detect interactions with epitope-tagged hSnf7-1 or AIP1 in Cos1 cell extracts (data not shown). Thus, our GST affinity-capture approach could detect only one of the known homologous yeast interactions, that of the interaction between hSnf7 proteins and AIP1. Additional immunoprecipitation studies showed that AIP1 interacted *in vivo* with Snf7 protein (data not shown).

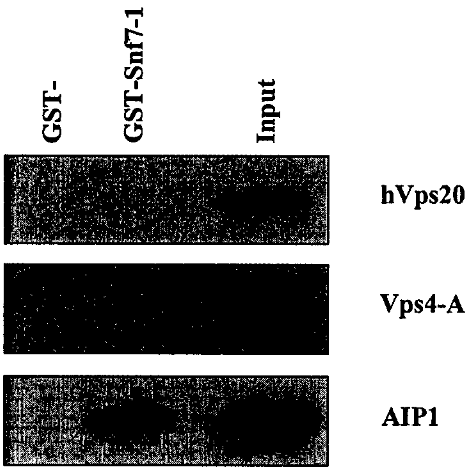

Figure 5 **hSnf7-1 binding to candidate proteins as detected by GST-affinity chromatography** Recombinant constructs for epitope-tagged hVps20, Vps4-A, and AIP1 proteins were expressed in Cos1 cells, lysates prepared and tested for binding to immobilized GST and GST-hSnf7-1. Following washing, the amount of bound protein was analyzed by Western blot. One-fifteenth of the each of the protein extracts was also electrophoresed on each SDS-PAGE gel to show the input available for binding.

We next investigated the relationship between Snf7-1 and AIP1 in HeLa cells by immunofluorescence. When FLAG epitope-tagged AIP1 was expressed in HeLa cells, it exhibited a diffuse cytoplasmic localization, with no detectable vesicular staining (Fig. 6A and B). When AIP1 and hSnf7-1 were cotransfected in HeLa cells, some of the AIP1 colocalized with Snf7-1 in the late endosomal/lysosomal vesicles (Fig. 6C and D). Consistent with our binding data, expression of the N-terminus of AIP1 (AIP1-N-Δ1) also colocalized with hSnf7-1 (Fig. 6E and F). While expression of the C-terminus of AIP1 (AIP1-C-Δ1) either alone or when cotransfected with Snf7-1 did not colocalize with hSnf7-1, it did show a vesicular staining pattern (data not shown). Taken together these results suggest that AIP1 normally resides with hSnf7 proteins and functions as part of the MVB machinery.

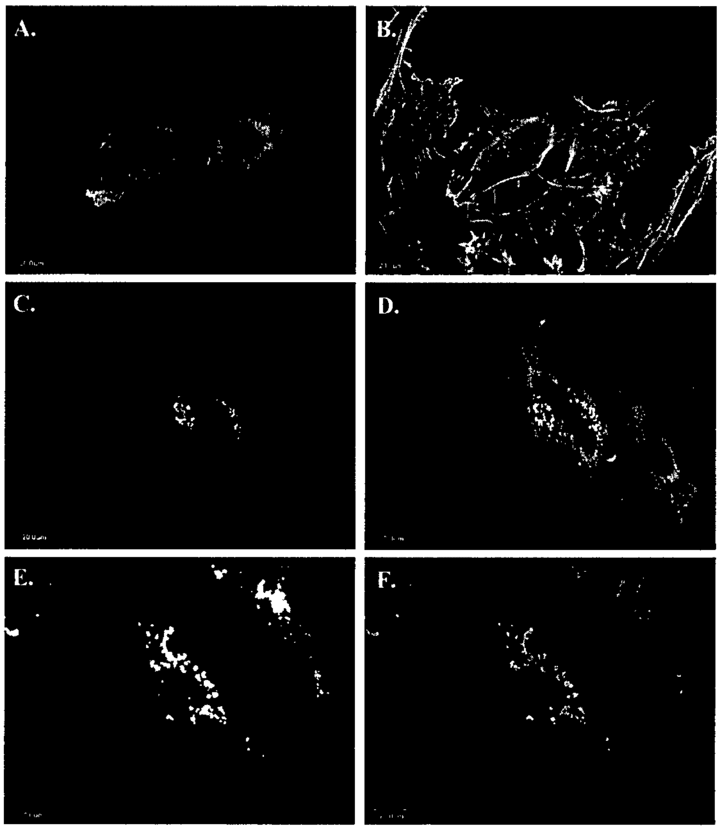

Figure 6 Vesicular colocalization of hSnf7-1 and AIP1. HeLa cells were transfected with FLAG-tagged AIP1 (A), cotransfected with Myc epitope-tagged hSnf7-1 (C) and FLAG-tagged AIP1 (D), Myc epitope-tagged hSnf7-1 (E) and FLAG-tagged AIP1-N-Δ1 (F). Twenty-four hours post-transfection, expressing cells were fixed and treated with anti-epitope antibodies followed by staining with FITC-conjugated goat anti-rabbit and Texas Red-conjugated goat anti-mouse antibodies. Actin was visualized by staining with Texas Red-conjugated phalloidin (B).

Studies with UEV3, a new component of the MVB pathway?

Previously our laboratory discovered a short splice variant of a TSG101 like molecule. Recent studies indicate that this molecule, renamed UEV3, contains a N-terminal UEV domain and a C-terminal region similar to the N-terminus of lactate dehydrogenase (LDH) A and B (Fig. 7) (24). In TSG101, the N-terminal UEV domain interacts with PTAP sequences in several cellular proteins including Hrs (10,11), AIP1 (12-14) and the viral late GAG protein, P6 of HIV1 (5,9). While the UEV domain of UEV3 is only 40% identical to that of TSG101 (24), most of the amino acid residues in TSG101 involved in binding to the PTAP sequence (9) are completely conserved with UEV3 (data not shown).

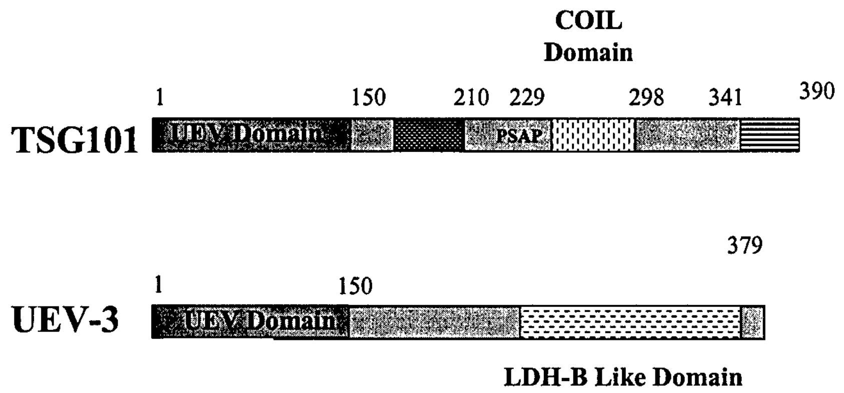

Figure 7. **Schematic of UEV-3 shares homology with TSG101.** TSG101 and UEV-3 encode 390 and 379 amino acid proteins, respectively. The N-terminal UEV domains of TSG101 and UEV-3 are 40% identical. While the C-terminal region of TSG101 contains a PSAP motif and a coil domain, UEV-3 contains an LDH-B like domain.

To determine if UEV3 interacts with PTAP sequences, we tested whether UEV3 interacts with P6 protein of HIV1. In these experiments, we produced soluble, recombinant MBP fusion proteins to the N-terminal UEV domain (MBP-UEV3-Δ1; amino acids 2-145), the C-terminal LDH-like domain (MBP-UEV3-Δ2; amino acids 2-145), or as a positive control to TSG101 (MBP-TSG101-Δ1; amino acids 2-145). Using these fusion proteins, we tested their interactions with GST, GST-P6 and GST-P6-P32A (a point mutant within the PTAP sequence which results in deficient TSG101 binding (5,6). Unfortunately, MBP-TSG101-Δ1, MBP-UEV3-Δ1 and MBP-UEV3-Δ2 showed non-specific binding to GST (data not shown), making it impossible to identify any specific interactions using this expression system. Future experiments using alternative techniques such as yeast two-hybrid assay and a *Renilla* luciferase-based protein-protein assay may be used in the future.

We next investigated the relationship between UEV3 and LDH in HeLa cells by immunofluorescence. When FLAG epitope-tagged LDH-A was expressed in HeLa cells, it exhibited a cytoplasmic staining pattern (data not shown). In contrast, overexpression of UEV3 showed a vesicular localization (Fig. 8), which appeared similar to the staining pattern of TSG101. Taken together these results suggest that UEV3 may normally reside on endosomes and recruit LDH isoenzymes to these structures for destruction in the lysosomes via the MVB machinery.

Figure 8. **UEV-3 localizes to vesicular-like structures.** EGFP-tagged UEV3 was expressed in HeLa cells for 48 hrs, the cells were fixed and analyzed by direct immunofluorescence. EGFP-UEV3 consistently demonstrated a vesicular staining pattern within the cytosol. A similar staining pattern was observed with TSG101, a known Escort-I component (data not shown).

In light of the role of TSG101 in recognizing monoubiquinated targets, we sought to identify whether LDH enzymes were ubiqutinated. To test this, LDH-A and LDH-B were overexpressed with HA-tagged ubiquitin in Cos1 cells under normal oxygen conditions. Following growth in normal oxygen conditions, both LDH-A and LDH-B were expressed at similar levels at approximately 38 kDa (data not shown). Parallel Western blot analysis for the incorporation of ubiquitin in LDH enzymes revealed that only the anaerobic isoform, LDH-A, was ubiquitinated (data not shown). Future experiments are needed to confirm these results, but are consistent with monoubiquitation since as a size shift of approximately 10 kDa was observed.

Studies with SPECs in immune function.

I have further characterized the role of SPECs in immune function (26). In these studies, SPEC1 and SPEC2 are structurally similar Cdc42-binding proteins of 79 and 84 amino acid residues, respectively. We investigated the role of SPEC2 in T cell function due to its gene location within the human cytokine cluster region at 5q31 and its high mRNA expression in lymphocytes. Western blot analysis revealed abundant SPEC2 protein in lymphocytes, which in GST-capture experiments specifically interacted with only GTP-bound Cdc42. Immunofluorescence experiments revealed that the SPEC2 protein was diffusely localized in the cytoplasm and at the cell membrane in unstimulated Jurkat T cells and Raji B cells. Recruitment of SPEC2 within Jurkat T cells to the APC interface occurred following incubation with SEE superantigenloaded B cells and colocalized there F-actin and Cdc42. TCR activation studies using anti-CD3 antibody-coated polystyrene beads showed that SPEC2 was recruited to the site of bead contact, which was not observed with anti-MHC antibody-coated beads. Accumulation of SPEC2 following TCR engagement occurred as early as 5 min, before obvious F-actin accumulation. Biochemical studies with Jurkat T cells demonstrated that N-terminal cysteine residues in SPEC2 were palmitoylated. Overexpression studies of the related SPEC1 showed that it also was recruited to the activated TCR. Mutational analysis revealed that localization of SPEC1 to the TCR required two N-terminal cysteine residues. Furthermore, a SPEC1 CRIB mutant, containing an intact N-terminus but defective in Cdc42 binding, completely blocked F-actin accumulation at the activated TCR. Taken together these results suggest that SPECs may play important roles in Cdc42-mediated F-actin accumulation at the immunological synapse.

III. Key research accomplishments funded by this Predoctoral DOD award

- We have cloned and characterized 3 human Snf7 and 1 VPs20 gene.
- Snf7overexpression disrupts MVB trafficking including disrupting the normal movement of cholesterol through this pathway.
- GST-capture experiments show that Snf7 interacts with AIP1.
- Immunoprecipitation experiments show that SNF7 and AIP1 interact *in vivo.*
- UEV3 colocalizes like TSG101 to MVB endosomes.
- We have found that UEV3 may function in regulating LDH monoubqiutination.
- Additional studies on SPECs show that they function in immune function.

IV. REPORTABLE OUTCOMES

Degree: Jeremy Peck received his Ph.D. in July, 2003 and was funded in part by this DOD award. Kathryn Ching hopes to receive her Ph.D. in Biochemistry and Molecular Biology in December, 2005, in part funded through this DOD fellowship.

Publications:

Peck, J.W. ***, Bowden, E.T., and Burbelo, P.D. Structure and function of human Vps20 and Snf7 proteins. Biochemical J. 377:693-700, 2004. (*** original holder of this DOD grant, which was funded by this award).

Kisailus, A.E., **Ching, K. H.**, Pirone, D.M. and Burbelo, P.D. Cdc42 regulates diverse signaling pathways in mammalian cells. In Recent Advances in Cell Research, Research Signpost 1: 161-180, 2003.

Burbelo, P.D., Pirone, D.M. and **Ching, K.H.** Global genomic approaches to the study of human gene function. Current Genomics 5:567-574, 2004.

Ching, K.H., Kisailus, A.E. and Burbelo, P.D. The role of SPECs, small Cdc42-binding proteins, in F-actin accumulation at the immunological synapse. J. Biol. Chem., published online April 19, 2005 as doi:10.1074/jbc.M500128200.

Burbelo, P.D. and **Ching, K.H.** Analyzing Protein Signaling Networks by Immunoprecipitation of Renilla-tagged Fusion Proteins (in preparation, 2005).

Ching, K.H., Kisailus, A.E. and Burbelo, P.D. Functional Domains of SPECs and Their Role in Phagocytosis (in preparation, 2005).

Poster Presentations and Seminars:

2003-2005 Seminars in the Department of Biochemistry and Molecular Biology, GUMC.

2004 Coauthor on a poster presented at the American Society of Cell Biology, Washington, DC.

2005 Poster Presentation at the Conference for Infectious Disease, Washington, DC

2005 Poster presentation at the annual Georgetown Research fair, GUMC.

- 2005 Poster presentation at the annual Lombardi Comprehensive Cancer Center Research fair, GUMC.

Will be presenting a poster at an Era of Hope Meeting in Philadelphia, Penn in June, 2005.

V. CONCLUSIONS

The data presented here provides insight into the roles of Vps20, AIP1 and Snf7 proteins in the MVB pathway. Coordinated regulation of protein trafficking by the components of the MVB pathway has been shown to be important for not only receptor down-regulation, but also for propagation of infectious agents such as HIV. The ability of Snf7, shown here, to recruit AIP1 to vesicular structures is of particular interest in that AIP1 is a necessary component of HIV viral buds. Taken together, our observations suggest that Snf7 proteins play an important role in MVB protein trafficking.

From my Ph.D. studies funded by this DOD fellowship, I have learned a variety of cellular and molecular biology skills. I have worked in the field of protein trafficking and immune function. My current goal is to obtain my Ph.D. Ultimately, I hope to fulfill my career goals of obtaining a medical degree and working in both the clinical and research fields of oncology.

VI. REFERENCES

1. Katzmann, D. J., Odorizzi, G., and Emr, S. D. (2002) *Nat Rev Mol Cell Biol* **3**, 893-905
2. Raymond, C. K., Howald-Stevenson, I., Vater, C. A., and Stevens, T. H. (1992) *Mol Biol Cell* **3**, 1389-1402
3. Babst, M., Katzmann, D. J., Snyder, W. B., Wendland, B., and Emr, S. D. (2002) *Dev Cell* **3**, 283-289
4. Babst, M., Katzmann, D. J., Estepa-Sabal, E. J., Meerloo, T., and Emr, S. D. (2002) *Dev Cell* **3**, 271-282
5. Garrus, J. E., von Schwedler, U. K., Pornillos, O. W., Morham, S. G., Zavitz, K. H., Wang, H. E., Wettstein, D. A., Stray, K. M., Cote, M., Rich, R. L., Myszka, D. G., and Sundquist, W. I. (2001) *Cell* **107**, 55-65
6. Martin-Serrano, J., Zang, T., and Bieniasz, P. D. (2001) *Nat Med* **7**, 1313-1319
7. Timmins, J., Schoehn, G., Ricard-Blum, S., Scianimanico, S., Vernet, T., Ruigrok, R. W., and Weissenhorn, W. (2003) *J Mol Biol* **326**, 493-502
8. Licata, J. M., Simpson-Holley, M., Wright, N. T., Han, Z., Paragas, J., and Harty, R. N. (2003) *J Virol* **77**, 1812-1819
9. Pornillos, O., Alam, S. L., Davis, D. R., and Sundquist, W. I. (2002) *Nat Struct Biol* **9**, 812-817
10. Lu, Q., Hope, L. W., Brasch, M., Reinhard, C., and Cohen, S. N. (2003) *Proc Natl Acad Sci U S A* **100**, 7626-7631
11. Pornillos, O., Higginson, D. S., Stray, K. M., Fisher, R. D., Garrus, J. E., Payne, M., He, G. P., Wang, H. E., Morham, S. G., and Sundquist, W. I. (2003) *J Cell Biol* **162**, 425-434
12. von Schwedler, U. K., Stuchell, M., Muller, B., Ward, D. M., Chung, H. Y., Morita, E., Wang, H. E., Davis, T., He, G. P., Cimbora, D. M., Scott, A., Krausslich, H. G., Kaplan, J., Morham, S. G., and Sundquist, W. I. (2003) *Cell* **114**, 701-713
13. Martin-Serrano, J., Yaravoy, A., Perez-Caballero, D., and Bieniasz, P. D. (2003) *Proc Natl Acad Sci U S A* **100**, 12414-12419
14. Strack, B., Calistri, A., Craig, S., Popova, E., and Gottlinger, H. G. (2003) *Cell* **114**, 689-699
15. Peck, J. W., Bowden, E. T., and Burbelo, P. D. (2003) *Biochem J* **Pt**
16. Katoh, K., Shibata, H., Suzuki, H., Nara, A., Ishidoh, K., Kominami, E., Yoshimori, T., and Maki, M. (2003) *J Biol Chem*
17. Chatellard-Causse, C., Blot, B., Cristina, N., Torch, S., Missotten, M., and Sadoul, R. (2002) *J Biol Chem* **277**, 29108-29115
18. Bishop, N., and Woodman, P. (2000) *Mol Biol Cell* **11**, 227-239
19. Uetz, P., Giot, L., Cagney, G., Mansfield, T. A., Judson, R. S., Knight, J. R., Lockshon, D., Narayan, V., Srinivasan, M., Pochart, P., Qureshi-Emili, A., Li, Y., Godwin, B., Conover, D., Kalbfleisch, T., Vijayadamodar, G., Yang, M., Johnston, M., Fields, S., and Rothberg, J. M. (2000) *Nature* **403**, 623-627
20. Ito, T., Chiba, T., Ozawa, R., Yoshida, M., Hattori, M., and Sakaki, Y. (2001) *Proc Natl Acad Sci U S A* **98**, 4569-4574
21. Babst, M., Wendland, B., Estepa, E. J., and Emr, S. D. (1998) *Embo J* **17**, 2982-2993
22. Missotten, M., Nichols, A., Rieger, K., and Sadoul, R. (1999) *Cell Death Differ* **6**, 124-129
23. Vito, P., Pellegrini, L., Guiet, C., and D'Adamio, L. (1999) *J Biol Chem* **274**, 1533-1540
24. Kloor, M., Bork, P., Duwe, A., Klaes, R., von Knebel Doeberitz, M., and Ridder, R. (2002) *Biochim Biophys Acta* **1579**, 219-224
25. Pornillos, O., Alam, S. L., Rich, R. L., Myszka, D. G., Davis, D. R., and Sundquist, W. I. (2002) *Embo J* **21**, 2397-2406
26. Ching, K. H., Kisailus, A. E., and Burbelo, P. D. (2005) *J Biol Chem*